REMARKS

I. Status of the Claims

Claims 58, 61-109 and 113-118 are pending in the present application. Claim 70 has been amended to recite that the splice donor site is 5′ to the negative selectable marker. Support for this amendment can be found in Applicants' specification, *inter alia*, on page 79:20-26.

Claims 79-84 and 87-98 have been amended to recite that the cells are eukaryotic. Support for this amendment can be found throughout Applicants' specification, for example on page 8:4.

Claim 96 has been amended to clarify that the cDNA is sequenced and compared to vector sequence.

Claim 97 has been amended to delete the term "such that" and directly recite that the splice donor is upstream of the first selectable marker and when the vector integrates into the genome, this results in splicing from the unpaired splice donor site to a splice acceptor site in the genome.

Claim 100 has been amended to point out how the endogenous gene sequence is used to recover exon I of an endogenous gene. Support for this amendment can be found in Applicants' specification, *inter alia*, in the paragraph spanning pages 83-84.

Claim 117 is amended to recite that the splice donor is within the first selectable marker and when the vector is integrated into the genome, this results in splicing from the splice donor

to a splice acceptor in the genomic DNA, thereby resulting in the first selectable marker being expressed in inactive form.

Claim 118 has been amended to recite that the splice donor site is within the negative selectable marker and that the negative selectable marker is expressed in inactive form because of the splicing event. Support for this amendment can be found in Applicants' specification, *inter alia*, on page 79:20-26.

New claim 119 is sought to be added. This claim is based on previous claim 100. Claim 100 has been amended to recite that the exon sequence in the endogenous gene is used to obtain a cellular transcript or a cDNA from a cellular transcript that contains the endogenous exon sequence and exon I of the endogenous gene. This claim originally encompassed also recovering exon I from the genomic DNA. Rather than lengthen claim 100, a new claim 119 has been written which is directed to claim 100 and to the aspect wherein the exon I that is obtained is in genomic DNA rather than based on a transcript. Support is, *inter alia*, in Applicants' specification in the paragraph spanning pages 83-84.

Accordingly, no new matter has been added with these amendments.

II. The Interview

Applicants thank Examiner Nguyen for the helpful interview held with Applicants' attorney, Anne Brown, on October 4, 2002. In this interview, the double patenting rejection was discussed. For the Examiner's convenience, Applicants provide a copy of the original Restriction Requirement and the claims that are the subject of the double patenting rejection. Applicants also discussed the enablement of claim 18 in which a splice donor site can be found in the coding region. Applicants' attorney explained that the splice donor sequence can remain in coding exons and not act as a mutagen but that, in any event, the claims were amended to clearly recite that mis-expression is not from the splice donor *per se* acting as an insertional mutagen, but results from the loss of nucleic acid sequence spliced out of the marker. The Examiner suggested that Applicants explain this in detail. Accordingly, this explanation is found in section IIIA.

In the interview, Applicants also discussed claim 100(g). Applicants submitted a proposed amendment to the claim for the interview. The Examiner indicated that the claim was acceptable but that it should indicate that the transcript or cDNA from the transcript contains exon I. Accordingly, Applicants have made every effort to take the Examiner's suggestions and address the issues as appropriate.

III. The Rejections

A. The Rejection Under 35 U.S.C. § 112, First Paragraph

On page 2 of the Office Action, claims 70-96, 106-107, 113-116 and 118 have been rejected under 35 U.S.C. § 112, first paragraph, on the grounds that the claims are not enabled. Applicants respectfully traverse the rejection.

One specific rejection regards claim 70. The Examiner asserts that the claim is enabled only "wherein said unpaired splice donor site is located 5′ upstream of said negative selectable marker encoding DNA sequence". Underline in original. Applicants have taken the Examiner's suggestion in the claims wherein, when splicing occurs, the gene is not expressed. However, as explained herein below, claim 118 encompasses an embodiment in which the splice donor is within the negative selectable marker. This means that part of the marker could be transcribed and translated but, as a result of splicing, transcription and translation does not result in functional expression of the negative selectable marker.

On page 4 of the Office Action, the Examiner states that the disclosure fails to teach how to use a vector construct where the negative selectable marker is inactive by the introduction of a splice donor site in the coding sequence. The Examiner states that when this is done, it is irrelevant whether the vector is integrated and spliced because the negative selectable marker will be inactivated by introducing the splice donor site. Applicants do not agree.

Introducing a splice donor site into a coding sequence does not necessarily inactivate the gene having the coding sequence. Coding exons contain splice donor sites which become part of

the finally translated protein. Accordingly, these sites themselves can have coding potential. It may be that the insertion of a splice donor site at a particular place in the negative selectable marker would *per se* inactivate the negative selectable marker function. However, genetic engineering was sufficiently developed at the time of Applicants' filing so that the person of ordinary skill could have ascertained by routine trial and error whether the insertion of a splice donor site into a negative selectable marker would, by itself, have inactivated negative selectable marker expression. These techniques need not be taught in Applicants' specification since splice donor sites were well-known and random insertion of splice donor sites into any sequence by routine ligation techniques could have been done by the person of ordinary skill in the art. Then, having ascertained the constructs in which the insertion did not itself destroy negative marker function, the artisan could then make a vector where inactivation would occur by means of the splicing reaction, and not the insertion *per se*.

As discussed above, in the interview, Applicants explained how splice donor sequences remain in coding exons but do not act as mutagens. Claim 118 encompassed mis-expression of a negative selectable marker, not the result of the splice donor site acting as an insertional mutagen, but as the result of splicing. The marker would not be functional because coding sequence required for functional expression has been spliced out of the transcript. The claim now recites that the lack of active expression is the result of splicing, not as the result of the marker merely containing a splice donor site (i.e., inactivation merely as a result of an insertional mutagenesis event).

Second, the Examiner asserts that the claims are enabled only for eukaryotic host cells. Accordingly, the claims have been amended to recite this limitation.

In view of these amendments, Applicants submit that the grounds of rejection have all been addressed and the rejection overcome. Reconsideration and withdrawal of the rejection is, therefore, respectfully requested.

B. **Rejection Under 35 U.S.C. § 112, Second Paragraph**

On page 6 of the Office Action, claims 70-100, 102-105, 108-109 and 113-118 are rejected under 35 U.S.C.§ 112, second paragraph, on the grounds that they are indefinite. Applicants respectfully traverse the rejection.

The Examiner states that in claim 96 the phrase "said isolation is accomplished by sequencing" is unclear because sequencing is not an "isolating" step. Accordingly, the claim has been amended to recite sequencing the cDNA and comparing the nucleotide sequence of the cDNA to the nucleotide sequence of the vector.

On page 6 of the Office Action, the Examiner states that, with respect to claims 70, 97, 117 and 118, the phrase "such that" renders the claims indefinite because it is unclear whether the limitations that follow the phrase are part of the claimed invention. Accordingly, the claims have been amended to recite that the limitations following the phrase are part of the claimed invention.

On page 7 of the Office Action, claim 100 is rejected on the grounds that "using" is not an active step. The Examiner questions what active steps are involved in using the endogenous gene sequence to recover exon 1 of the endogenous gene. This was specifically discussed in the interview. The Examiner indicated that Applicants' proposed claim, with an amendment in step (g), would be acceptable as long as the claim indicated that the transcript or cDNA from the transcript contains exon I. Accordingly, the proposed claim, with the Examiner's suggested amendment, is presented herein.

On page 7 of the Office Action, claims 102 and 103 are rejected for reciting "one or more genes encoded by said vector " in step (d). The rationale of the Examiner is that the vectors do not contain one or more genes for transcription in step (d). Applicants point out that the vector can contain genes that are transcribed. For example, the vector of claim 58 contains, at the very least, a selectable marker that can be transcribed. Since, however, according to the Examiner's suggestion, the vector may also contain other transcribed sequences, the phrase has been amended to generally recite "one or more nucleic acid sequences in said vector". This is understood to encompass entire gene sequences as well as subsequences.

In view of these amendments, Applicants submit that the grounds of rejection have all been addressed and the rejection overcome. Reconsideration and withdrawal of the rejection is, therefore, respectfully requested.

C. **Double Patenting**

On page 8 of the Office Action, claims 58, 61-109 and 113-118 have been rejected as being unpatentable over claims 1-58 of U.S. Patent No. 6,361,972. Applicants traverse the rejection.

This rejection is improper because these claims were restricted into two different groups in a Restriction Requirement issued in Applicants' parent case, 09/276,820 . The claims in the present case were placed in Group XIII. The claims in U.S. 6,361,972, were placed in Group X. A copy of the Restriction Requirement, with the designated groups, is attached for the Examiner's convenience (**Exhibit 1**). Independent claims in each group are also attached for the Examiner's convenience (**Exhibit 2**). The claims in Group X were renumbered and submitted in a divisional application as were claims in the present divisional application. Accordingly, the Examiner can see the difference in the vectors in these two groups and can also see that it was a conclusion of the U.S. Patent and Trademark Office that the claims in these two groups were patentably distinct. The Restriction Requirement was not withdrawn. Accordingly, a double patenting rejection at this time is improper and should be withdrawn.

In view of these amendments, Applicants submit that the grounds of rejection have all been addressed and the rejection overcome. Reconsideration and withdrawal of the rejection is, therefore, respectfully requested.

Accordingly, Applicants believe that the application is in condition for allowance. Early notification in that regard is requested. If the Examiner believes that a telephonic interview

would expedite prosecution of this case, he is invited to contact Applicants' attorney, Anne Brown, at 216-426-3586 or Joseph Contrera at 703-683-6197.

Attached hereto is a marked-up version of the changes made to the claims by the current amendment. The attached page is captioned **"Amended Claims with Marking to Show Changes Made"**.

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VERSION WITH MARKINGS SHOWING CHANGES MADE

In the Claims:

61. (Once amended) The vector of claim 58 ~~59~~, wherein said vector is linear and wherein said second promoter is located 5' to said unpaired splice donor site.

70. (Twice amended) A vector construct comprising:

(a) a first promoter operably linked to a sequence encoding a positive selectable marker;

(b) a second promoter operably linked to a sequence encoding a negative selectable marker; and

(c) an unpaired splice donor site,

wherein said splice donor site is 5′ to said negative selectable marker ~~said positive and negative selectable marker sequences and said splice donor site are oriented in said vector construct in an orientation such that,~~ and when said vector construct is integrated into the genome of a eukaryotic host cell and the vector-encoded splice donor is spliced to a splice acceptor in an endogenous gene in said genome, then said positive selectable marker sequence is expressed in active form and said negative selectable marker sequence is not expressed.

79. (Twice amended) A eukaryotic host cell *in vitro* comprising the vector of any one of claims 58, 65, 67, 70, or 71.

80. (Twice amended) A eukaryotic host cell *in vitro* comprising the vector of claim 72.

81. (Twice amended) A eukaryotic host cell *in vitro* comprising the vector of claim 73.

82. (Twice amended) A eukaryotic host cell *in vitro* comprising the vector of claim 74.

83. (Twice amended) A eukaryotic host cell *in vitro* comprising the vector of claim 75.

84. (Twice amended) A eukaryotic host cell *in vitro* comprising the vector of claim 78.

85. (Once amended) The eukaryotic host cell of claim 79, wherein said host cell is an isolated cell.

86. (Once amended) The eukaryotic host cell of any one of claims 80-85, wherein said host cell is an isolated cell.

87. (Twice amended) A library of eukaryotic cells *in vitro* comprising the vector of any one of claims 58, 65, 67, 70, or 71.

88. (Twice amended) A library of eukaryotic cells *in vitro* comprising the vector of claim 72.

89. (Twice amended) A library of eukaryotic cells *in vitro* comprising the vector of claim 73.

90. (Twice amended) A library of eukaryotic cells *in vitro* comprising the vector of claim 74.

91. (Twice amended) A library of eukaryotic cells *in vitro* comprising the vector of claim 75.

92. (Twice amended) A library of eukaryotic cells *in vitro* comprising the vector of claim 78.

93. (Twice amended) A method for activation of an endogenous gene in a eukaryotic cell *in vitro* comprising:

(a) transfecting a eukaryotic cell *in vitro* with the vector of any one of claims 58, 65, 67, 70, or 71; and

(b) culturing said cell under conditions suitable for non-homologous integration of said vector into the genome of said cell, wherein said

integration results in the activation of an endogenous gene in the genome of said cell.

94. (Twice amended) A method for obtaining cDNA from an endogenous gene comprising:

(a) transfecting a plurality of eukaryotic cells *in vitro* with the vector of any one of claims 58, 65, 67, 70, or 71;

(b) culturing said cells under conditions suitable for non-homologous integration of the vector into the genome of the cell;

(c) selecting for cells in which said vector has integrated into the genomes of said cells;

(d) isolating RNA from said selected cells;

(e) producing cDNA from said isolated RNA; and

(f) isolating one or more cDNA molecules containing one or more nucleotide sequences from said vector.

96. (Twice amended) The method of claim 94, wherein ~~said isolation is accomplished by sequencing~~ said cDNA is sequenced and ~~comparing~~ the nucleotide sequence of said cDNA is compared to ~~the~~ nucleotide sequence in ~~of~~ said vector.

97. (Twice amended) The vector of claim 67, wherein said unpaired splice donor site is positioned upstream of said first selectable marker sequence and ~~such that,~~ when said vector is integrated into the genome of a eukaryotic host cell resulting in splicing from said unpaired splice donor site to a genome-encoded splice acceptor site, then said first selectable marker sequence is not expressed.

98. (Twice amended) A method for isolating eukaryotic cells *in vitro* in which a single exon gene has been activated, comprising:

(a) transfecting a plurality of eukaryotic cells *in vitro* with the vector of claim 97;

(b) culturing said cells under conditions suitable for non-homologous integration of the vector into the genomes of said cells; and

(c) selecting for cells in which said first and second selectable marker sequences are expressed in their active forms.

100. (Twice amended) A method for isolating exon I of a gene comprising:

(a) transfecting one or more eukaryotic cells *in vitro* with the vector of any one of claims 58, 61, 65, or 67;

(b) culturing said cells under conditions suitable for non-homologous integration of the vector into the genome of said cells;

(c) selecting for cells in which said vector has transcriptionally activated an endogenous gene containing one or more exons;

(d) isolating RNA from said selected cells;

(e) producing cDNA from said isolated RNA;

(f) recovering a cDNA molecule containing vector sequence and exon sequence from said endogenous gene; and

(g) using the exon sequence in the endogenous gene in (f) to obtain a cellular transcript or cDNA of a cellular transcript that contains the endogenous gene exon sequence and exon I of the endogenous gene. ~~using said endogenous gene sequence to recover exon I of said endogenous gene.~~

102. (Twice amended) A method for producing a gene product encoded by genomic DNA ~~an endogenous cellular genomic gene,~~ comprising:

(a) isolating genomic DNA, containing at least one gene, from a eukaryotic cell;

(b) inserting into or otherwise combining with said isolated genomic DNA, the vector of any one of claims 58, ~~59,~~ 61, 65, or 67, thereby producing a vector-genomic DNA complex;

(c) transfecting said vector-genomic DNA complex into a suitable eukaryotic host cell *in vitro*; and

(d) culturing said host cell under conditions suitable to result in transcription of one or more nucleic acid sequences in ~~genes encoded by~~ said vector contained in said vector-genomic DNA complex.

103. (Twice amended) A method for isolating a gene sequence comprising:

(a) isolating genomic DNA, containing at least one gene, from a eukaryotic cell;

(b) inserting into or otherwise combining with said isolated genomic DNA, the vector of any one of claims 58, 61, 65, or 67, thereby producing a vector-genomic DNA complex;

(c) transfecting said vector-genomic DNA complex into a suitable eukaryotic host cell *in vitro*;

(d) culturing said host cell under conditions suitable to result in transcription of one or more nucleic acid sequences in ~~genes encoded by~~ said vector contained in said vector-genomic DNA complex;

(e) isolating RNA produced by said transcription from said host cell;

(f) producing one or more cDNA molecules from said isolated RNA; and

(g) recovering one or more cDNA molecules containing vector sequences at the 5' ends of said cDNA molecules, thereby isolating said gene sequence.

117. (Once amended) The vector of claim 67, wherein said unpaired splice donor site is positioned within said first selectable marker sequence and ~~such that,~~ when said vector is integrated into the genome of a eukaryotic host cell resulting in splicing from said unpaired splice donor site to a genome-encoded splice acceptor site, then said first selectable marker sequence is expressed in inactive form.

118. (Once amended) A vector construct comprising:

(a) a first promoter operably linked to a sequence encoding a positive selectable marker;

(b) a second promoter operably linked to a sequence encoding a negative selectable marker; and

(c) an unpaired splice donor site,

wherein said splice donor site is within said negative selectable marker and ~~said positive and negative selectable marker sequences and said splice donor site are oriented in said vector construct in an orientation such that,~~ when said vector construct is integrated into the genome of a eukaryotic host cell and the vector-encoded splice donor is spliced to a splice acceptor in an

endogenous gene in said genome, then said positive selectable marker sequence is expressed in active form and said negative selectable marker sequence is expressed in inactive form because of the splicing event.

Application/Control Number: 09/276,820

Art Unit: 1632

DETAILED ACTION

1. Claims 1-7, 10-15, 20-36 and 58-231 are pending in the instant application

Election/Restriction

2. Restriction to one of the following inventions is required under 35 U.S.C. 121:

I. Claims 1-4, 10-12, 15, 20-24, 28-29, 85, 86, 128, 159, 161-162, 164-167, 169-175, 177-183, and 214, drawn to a vector and uses thereof, classified in class 435, subclass 320.1.

II. Claims 5-7, 13-14, 20-24, 28-29, 85, 128, 159, 161-162, 164-167, 169-175, 177-183, and 227, drawn to a vector and uses thereof, classified in class 435, subclass 320.1.

III. Claims 1-4, 10-12, 15, 20-24, 28-29, 85, 86, 128, 159, 161-162, 164-167, 169-175, 177-183, and 214, drawn to a vector and uses thereof, classified in class 435, subclass 320.1.

IV. Claims 5-7, 13-14, 20-24, 28-29, 85, 128, 159, 161-162, 164-167, 169-175, 177-183, and 227, drawn to a vector and uses thereof, classified in class 435, subclass 320.1.

V. Claims 30-35, drawn to primers, classified in class 536, subclass 24.33.

VI. Claims 36, drawn to a method of DNA synthesis, classified in class 435, subclass 91.3.

VII. Claims 58, 59, 64-69, 71-74, 76-82, 85-123, 128, 129, 130-132, 157, 159, 161-162, 164-167, 169-175, 177-183, and 223-226, drawn to a vector and uses thereof, classified in class 435, subclass 320.1.

VIII. Claims 60, 63-86, 99-115, 128, 130-132, 197, 203, and 223-224, drawn to a vector and uses thereof, classified in class 435, subclass 320.1.

IX. Claims 61, 63-86, 99-115, 128, 130-132, and 223-224, drawn to a vector and uses thereof, classified in class 435, subclass 320.1.

X. Claims 62-82, 99-115, 128,130-132, 159, 161-162, 164-167, 169-175, 177-183, and 223-224, drawn to a vector and uses thereof, classified in class 435, subclass 320.1.

XI. Claims 124-127, and 129, drawn to a method of enhancing gene expression, classified in class 435, subclass 455.

XII. Claims 133-137, drawn to a method of identifying cells, classified in class 435, subclass 455.

XIII. Claims 138-144, 149-153, 157-188, 191-195, 198, 200-202, 204, 215-217 and 221, drawn to a vector and uses thereof, classified in class 435, subclass 320.1.

XIV. Claims 145-148, 159-183, 186-188, 191-195, 198, 200-202, 204, and 215-216, drawn to a vector and uses thereof, classified in class 435, subclass 320.1.

XV. Claims 154-156, 159-183, 186-188, 191-195, 198, 200-202, and 204, drawn to a vector and uses thereof, classified in class 435, subclass 320.1.

XVI. Claims 189-190, 196, 199, and 203, drawn to a method of gene expression, classified in class 435, subclass 455.

XVII. Claims 205-213, 218-220, and 221-222, drawn to a method of gene expression, classified in class 435, subclass 455.

XVIII. Claims 228-231, drawn to a method of drug discovery, classified in class 435, subclass 375.

3. Claims 1-4, 10-12 and 15 are generic to groups I and III. Should any of these groups be elected, claims 1-4, 10-12, and 15 will be examined to the extent they encompass the elected invention.

4. Claims 5-7 and 13-14 are generic to groups II and IV. Should any of these groups be elected, claims 5-7 and 13-14 will be examined to the extent they encompass the elected invention.

5. Claims 20-24 and 28-89 are generic to groups I - IV. Should any of these groups be elected, claims 20-24 and 28-89 will be examined to the extent they encompass the elected invention.

6. Claim 85 is generic to groups I-IV and VII-IX . Should any of these groups be elected, claim 85 will be examined to the extent it encompasses the elected invention.

Please insert the following new claims:

--58. A vector construct comprising a transcriptional regulatory sequence operably linked to an unpaired splice donor sequence and one or more amplifiable markers, wherein said vector construct does not comprise a homologous targeting sequence.

59. A vector construct comprising a transcriptional regulatory sequence, an amplifiable marker, and a viral origin of replication.

60. A vector construct comprising a selectable marker, a transcriptional regulatory sequence operably linked to a translational start codon, a secretion signal sequence, an epitope tag, and an unpaired splice donor site.

61. A vector construct comprising a transcriptional regulatory sequence operably linked to a translational start codon, a secretion signal sequence, an epitope tag, a sequence-specific protease site, and an unpaired spice donor site.

62. A vector comprising:

(a) a transcriptional regulatory sequence operably linked to a translation start codon,

(b) a nucleic acid sequence encoding an amino acid sequence of four or more amino acids, wherein said amino acid sequence alone is insufficient to constitute signal peptide activity, but is sufficient to constitute signal

peptide activity when said nucleic acid sequence is combined with or is upstream of an exon of an endogenous gene, and

(c) an unpaired splice donor site.

63. The vector construct of any one of claims 60-62, wherein said construct further comprises one or more amplifiable markers.

64. The vector construct of any of claims 58 and 60-62, wherein said transcriptional regulatory sequence is a promoter.

65. The vector construct of claim 64, wherein said promoter is a viral promoter.

66. The vector construct of claim 65, wherein said viral promoter is a cytomegalovirus immediate early gene promoter.

67. The vector construct of claim 65, wherein said promoter is a non-viral promoter.

68. The vector construct of claim 65, wherein said promoter is an inducible promoter.

69. A cell containing the vector construct of any one of claims 58-62.

70. A cell containing the vector construct of claim 63.

106. The method of claim 77, further comprising introducing double strand breaks into the genomic DNA of said cell prior to or simultaneously with integration of said vector.

107. A gene expression product produced by the method of any one of claims 83, 85-87, 89 and 98.

108. The method of any one of claims 83, 85-87, 89 and 98, wherein said vector construct is linear.

109. A method for producing an expression product of an endogenous gene in a cell comprising:

(a) introducing a vector comprising a transcriptional regulatory sequence into at least one isolated genome-containing cell;

(b) integrating said vector into the genome of said cell by non-homologous recombination;

(c) over-expressing an endogenous gene or a portion thereof in said cell by upregulation of said gene by said transcriptional regulatory sequence;

(d) screening said cell for over-expression of said endogenous gene; and

(e) culturing said cell in reduced serum medium.

110. A method of protein discovery comprising:

(a) introducing a vector comprising a transcriptional regulatory sequence into at least one isolated genome-containing cell;

(b) integrating said vector into the genome of said cell by non-homologous recombination;

(c) culturing said cell in reduced serum medium under conditions that allow over-expression of an endogenous gene or a portion thereof in said cell by upregulation of said gene by said transcriptional regulatory sequence, thereby producing cell-conditioned media; and

(d) screening said cell-conditioned media for the presence of the expression product of said gene or portion thereof.

111. The method of claim 110, further comprising concentrating said cell-conditioned media prior to screening in (d).

112. The method of any one of claims 109-111, wherein said method comprises a high-throughput assay.

113. A method for producing an expression product of an endogenous cellular gene comprising:

(a) introducing a vector comprising a transcriptional regulatory sequence into a cell;

(b) integrating said vector into the genome of said cell by non-homologous recombination;

(c) over-expressing an endogenous gene or a portion thereof in said cell by upregulation of said gene by said transcriptional regulatory sequence;

(d) screening said cell for over-expression of said endogenous gene; and

(e) culturing said cell under conditions favoring the production of the expression product of said endogenous gene by said cell; and

(f) isolating said expression product from a cell mass equivalent to at least 10 liters of cells at 10^4 cells/ml.

114. The method of any of claims 109-111 and 113, wherein said vector further comprises one or more amplifiable markers.

115. The method of any of claims 109-111 and 113, wherein said vector further comprises an unpaired splice donor site.

116. A method for increasing expression of an endogenous gene in a cell *in situ*, the phenotype of said gene being known, without making use of any sequence information of the gene, the method comprising the steps of:

(a) constructing a vector comprising an amplifiable marker, a transcriptional regulatory sequence, and an unpaired splice donor sequence;

(b) delivering copies of the vector to a plurality of cells;

(c) culturing the cells under conditions permitting nonhomologous recombination events between the inserted vector and the genome of the cells;

XIII

138. A vector comprising:

(a) a first promoter operably linked to an exon and an unpaired splice donor site, and

(b) a second promoter operably linked to a selectable marker lacking a polyadenylation signal.

139. The vector of claim 138, wherein said first and second promoters are present in said vector in the same orientation.

140. The vector of claim 139, wherein said vector is linear and wherein said selectable marker is located 3' to said first promoter.

141. The vector of claim 139, wherein said vector is linear and wherein said second promoter is located 5' to said unpaired splice donor site.

142. The vector of claim 138, wherein said exon lacks a translation start codon.

143. The vector of claim 138, wherein said exon comprises a translation start codon.

144. The vector of claim 138, wherein said exon comprises a translation start codon and a signal secretion sequence.

149. A vector comprising a first promoter and a second promoter, said first and second promoters being oriented in the same direction, wherein:

(a) said first promoter, but not said second promoter, is operably linked to an unpaired splice donor site; and

(b) said vector comprises no polyadenylation signals downstream of either said first promoter or said second promoter.

XIII

150. The vector of claim 149, wherein said vector is linear and wherein said second promoter is located 3' to said first promoter.

151. A vector comprising:

(a) a first promoter operably linked to a first selectable marker containing an unpaired splice donor site; and

(b) a second promoter operably linked to a second selectable marker,

wherein neither said first selectable marker nor said second selectable marker contains a polyadenylation signal.

XIII

152. The vector of claim 151, wherein said first and second selectable markers are positive selectable markers.

153. The vector of claim 151, wherein said first selectable marker is located upstream of said second selectable marker.

154. A vector comprising:

(a) a first promoter operably linked to a first exon and a first unpaired splice donor site; and

(b) a second promoter operably linked to a second exon and a second unpaired splice donor site,

wherein the nucleotide sequence of said first exon is different from the nucleotide sequence of said second exon.

155. The vector of claim 154, wherein said first and second exons each comprises a translation start codon and an open reading frame that does not terminate with a stop codon.

156. The vector of claim 154, wherein said first exon, said second exon, or both said first and second exons, lack a translation start codon.

157. A vector construct comprising:

(a) a first promoter operably linked to a positive selectable marker;

(b) a second promoter operably linked to a negative selectable marker; and

(c) an unpaired splice donor site,

XIII

wherein said positive and negative selectable markers and said splice donor site are oriented in said vector construct in an orientation that, when said vector construct is integrated into the genome of a eukaryotic host cell in such a way that an endogenous gene in said genome is transcriptionally activated, then said positive selectable marker is expressed in active form and said negative selectable marker is either not expressed or is expressed in inactive form.